CTS™ Essential 6 Medium

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Gibco™ CTS™ Essential 6 Medium is a fully-defined, xeno-free medium, which supports reprogramming of somatic cells and the differentiation of human pluripotent stem cells. CTS™ Essential 6 Medium requires the addition of basic fibroblast growth factor (bFGF) when reprogramming human cells.

Contents and storage

Contents	Amount	Storage	Shelf Life[1]
CTS™ Essential 6 Medium	500 mL	Store at 2–8°C. Protect from light	12 months

[1] Shelf Life duration is determined from Date of Manufacture.

Culture conditions

Media: CTS™ Essential 6 Medium

Cell line: Human pluripotent stem cells (PSCs)

Temperature range: 37°C

Incubator atmosphere range: Humidified atmosphere of 5% CO_2

Culture type: Adherent

Recommended culture vessels: Induced pluripotent stem cells (iPSCs) can be derived and/or differentiated in complete CTS™ Essential 6 Medium on CTS™ Vitronectin (VTN-N)-coated, tissue culture-treated vessels. Embryoid bodies (EBs) can be derived in CTS™ Essential 6 Medium using non-tissue culture-treated or low attachment vessels.

Use CTS™ Essential 6 Medium for embryoid body (EB) formation

1. Observe the human iPSCs growing in CTS™ Essential 8™ Medium under the microscope to confirm that the cells are 70–85% confluent and ready to be subcultured.
2. Pre-warm the required volume of CTS™ Versene Solution and CTS™ Essential 6 Medium to room temperature.
3. Aspirate the spent medium from the vessel containing PSCs, and rinse the cells with CTS™ DPBS without calcium chloride, without magnesium chloride.
4. Add CTS™ Versene Solution to the culture dish (e.g., 1 mL of CTS™ Versene Solution per well of a 6-well plate).

   Swirl the culture dish to coat the entire cell surface.
5. Incubate the vessel at room temperature for 5 to 8 minutes or at 37°C for 4 to 5 minutes.

   When the cells start to separate and round up, and the colonies appear to have holes in them when viewed under a microscope, they are ready to be removed from the vessel.
6. Aspirate the CTS™ Versene Solution and resuspend the cells in CTS™ Essential 8™ Medium containing CTS™ RevitaCell™ Supplement at a 1X final concentration (e.g. 2 mL CTS™ Essential 8™ Medium with 1X CTS™ RevitaCell™ Supplement per well of a 6-well plate).
7. Transfer cell solution to 1 or 2 wells of a non-tissue culture-treated 6-well plate.

   Ensure the final volume of CTS™ Essential 8™ Medium with 1X CTS™ RevitaCell™ Supplement is 2 mL per well.
8. Place the vessel in a 37°C incubator with a humidified atmosphere of 5% CO_2.
9. On the next day, replace the CTS™ Essential 8™ Medium with 1X CTS™ RevitaCell™ Supplement + CTS™ Essential 6 Medium. Gently transfer the cell solution to a 15-mL conical tube using a 5-mL sereological pipette to prevent breaking apart EBs.

   Keep the tube in the hood and allow the cells to settle to the bottom of the tube (about 5 minutes).

10. Remove the supernatant from the tube and replace it with CTS™ Essential 6 Medium.

    Place the cells back into the same vessel.

11. Continue to exchange the medium with fresh CTS™ Essential 6 Medium every other day.

12. Continue with downstream differentiation, or after 7–21 days, harvest EBs to assay for trilineage differentiaton potential.

    To assess trilineage potential, the EBs can be harvested and assayed using the TaqMan® hPSC Scorecard™ Kit.

Derive induced pluripotent stem cells (iPSCs) from fibroblasts in CTS™ Essential 6 Medium

Day –2: Two days before transduction, plate human neonatal foreskin fibroblast cells into two wells of a 6-well plate at the appropriate density to achieve 80–90% confluency per well on the day of transduction (Day 0).

Day 0: Perform transduction.

Day 1: 24 hours after transduction, replace the medium with fresh fibroblast medium. Culture the cells for 5 more days, changing the spent medium with fresh fibroblast medium every other day.

Day 6: Replace the medium with CTS™ Essential 6 Medium supplemented with bFGF (100 ng/mL).

Day 7: Harvest cells and seed on CTS™ Vitronectin-coated (1 µg/cm^2) plates using CTS™ Essential 6 Medium supplemented with bFGF (100 ng/mL); replace the spent medium every day thereafter.

Day 8 to 28: Feed and monitor the cells. When colonies are ready for transfer, perform live staining using TRA-1-60 or TRA-1-81 to select reprogrammed colonies. Manually pick colonies and transfer them onto prepared CTS™ Vitronectin-coated plates and culture them in CTS™ Essential 8™ Medium.

Note: Colonies are typically ready to be picked at Day 21, but they may require a few additional days depending on the somatic cell line.

Identify iPSC colonies

By Day 21 post-transduction, the cell colonies on the vitronectin-coated plates are large and compact, covering the majority of the surface area of the culture vessel. However, only a fraction of these colonies will consist of iPSCs, which exhibit a hESC-like morphology characterized by a flatter cobblestone-like appearance with individual cells clearly demarcated from each other in the colonies. Therefore, we recommend that you perform live staining with TRA-1-60 or TRA-1-81 antibodies that recognize undifferentiated iPSCs.

Pick iPSC colonies

1. Place the culture dish containing the reprogrammed cells under an inverted microscope and examine the colonies under 10X magnification.

2. Mark the colony to be picked on the bottom of the culture dish.

   Note: We recommend picking at least 10 distinct colonies by the end of each reprogramming experiment and expanding them in separate 24-well culture plates.

3. Transfer the culture dish to a sterile cell culture hood (i.e., biosafety cabinet) equipped with a stereomicroscope.

4. Use a 25-gauge 1½-inch needle to cut the colony to be picked into 5–6 pieces in a grid-like pattern.

5. Use a 200 µL pipette to transfer the cut pieces to one well of a freshly prepared 24-well CTS™ Vitronectin-coated culture plate containing human CTS™ Essential 8™ Medium.

6. Incubate the culture plate containing the picked colonies in a 37°C incubator with a humidified atmosphere of 5% CO_2.

7. Allow the colonies to attach to the culture plate for 48 hours before replacing the spent medium. After that, change the medium every day.

8. Treat the reprogrammed colonies like normal human ESC colonies and passage, expand, and maintain them using standard culture procedures until you have frozen cells from two 60-mm plates.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
CTS™ Essential 8™ Medium	A2656101
CTS™ Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A27940
CTS™ RevitaCell™ Supplement	A4238401
CTS™ CytoTune™ -iPS Sendai Reprogramming Kit	A34546
CTS™ Versene Solution	A42391
CTS™ DPBS without calcium chloride, without magnesium chloride	A12856
TaqMan® hPSC Scorecard™ Kit	A15872
FGF-Basic (AA 1-155) Recombinant Human	PHG0261

Limited product warranty

Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.